EFFECT OF MOLECULAR ASSOCIATION AND CHARGE DISTRIBUTION ON THE GELATION OF PECTIN[1]

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When a polymer solution gels, a liquid solution incapable of withstanding shear stress is transformed into a "solid" solution which is rigid and elastic. The requirement for such a transformation is the ability of the polymer molecules to form an extended three-dimensional network which is sufficiently rigid to support shear, yet is capable also of incorporating mobile solvent molecules within interstices of the structure. Two sorts of gels may be distinguished: (*1*) irreversible gels, stable to heat and additional solvent, in which the polymer molecules are cross-linked by covalent bonds; and (*2*) reversible gels, dispersible upon heating and usually soluble in excess solvent, in which cross-links are secondary valence or ionic bonds.

The definition of a gel of type 1 is not ambiguous. To illustrate, if vulcanized rubber is placed in benzene, it swells until the elastic reaction of the rubber gel

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just equals the osmotic pressure of the benzene, and gel and solvent achieve an equilibrium not affected by excess solvent. Heating upsets the equilibrium, changing the amount of benzene imbibed by the rubber, but the gel does not disperse.

Type 2 gels behave in a considerably more complex fashion. Recognizing the gel may sometimes be difficult, because the transition point between gel and solvated precipitate of the gelling agent is ill defined. Recourse to a purely thermodynamic definition of the gel is not satisfactory, because reactions that govern the formation and stability of these gels are often slow and as yet quantitatively unpredictable. For a polymeric gelling agent of this type, which is effective at concentrations less than 1 per cent and still yields gels rigid enough to be easily measured (as on a Delaware jelly-strength tester (14), the general requirement is a high molecular weight and a polymer surface capable of forming numerous intermolecular secondary valence bonds. In some substances—pectin, for example—dipole interaction leads to hydrogen bonding, and coulombic forces lead simultaneously to ionic bonding. In any event the polymer has only limited solubility in the solvent, so that although there are enough intermolecular contacts to form a rigid gel, the numerous voids and the high affinity of the solvent for the polymer make it possible for the gelling agent to contain a considerable volume of solvent. The number of intermolecular contacts necessary before a gel structure can be considered a precipitate is arbitrary, and depends upon the means used to define and measure gel properties.

Aqueous solutions of pectin and the pectinic acids give gels of type 2 above. The aggregative tendency of a pectic material depends upon its degree of esterification (λ) (3) and the nature of the aqueous solution used as solvent. It has been shown previously (1, 2, 4, 12) that two sub-types of pectic gels can be prepared: (*1*) a hydrogen-bonded gel, conventionally incorporating 65 per cent sugar; and (*2*) an ionic-bonded gel made with a multivalent ion such as Ca^{++}.[3] Optimum strength of hydrogen-bonded pectin and pectinic acid gels depends principally upon the molecular weight. However, optimum strength of calcium pectinate gels is influenced strongly also by the mode of pectin deësterification. Enzyme-deësterified pectinic acids make weaker gels than do acid- (4, 12) or alkali-deësterified pectinic acids (7). Table 1 shows that while Sample H89C (enzyme deësterified) had a higher molecular weight than H84D (acid deësterified), the H84D calcium gel was the stronger. Significantly, also, the enzyme-deësterified pectinic acid gave the stronger sugar gel (compare again H89C and H84D in table 1). In earlier communications (2, 12), this difference in gelling behavior was attributed to a difference in the homogeneity of λ among the molecules making up the pectinic acid samples, the enzyme-deësterified preparations being the less homogeneous.

In this paper, experimental evidence obtained by electrophoresis measurements

[3] Although calcium pectinate gels incorporating no sucrose or other hydrogen-bonding agent can be made, it is advantageous for some purposes to use both sucrose and calcium salt in the gel composition (4). In this paper the calcium pectinate gels ("calcium gels") contained 35 per cent sucrose, and the "sugar gels" contained 65 per cent sucrose.

that substantiates this hypothesis is given. In addition, pectin gel quality is correlated with data on turbidity, viscosity, and solubility and with the relation of pectinic acid solubility to the ionic equilibria among pectinic acid, calcium pectinate, hydrogen ions, and calcium ions.

TABLE 1

Strength of pectinic acid gels

SAMPLE	λ	MODE OF DEËSTERIFICATION	GEL STRENGTH (CM. OF WATER)		MOLECULAR WEIGHT*	
			Sugar gel	Calcium gel	M_w	M_n
H84	0.80	None	61	0	105,000	83,400
H84D	0.32	Acid	20	30	80,800	57,200
H89C	0.35	Enzyme	47	13	102,000	76,700
H85C	0.39	Acid	60	57		
H87C	0.36	Enzyme	135	52†		
H59	About 0.30	Acid	72	56	199,000	143,000
H74	About 0.30	Enzyme	24	4	162,000	64,400

* Molecular weight of the nitrate (12, 13). This molecular weight × 0.73 = molecular weight of the pectinic acid. M_w and M_n are the weight- and number-average molecular weights.

† All but the H87C calcium gel were made with 1 g. of pectinic acid. The H87C calcium gel was made with 2 g., in order to obtain a measurably strong calcium gel.

EXPERIMENTAL

Apple pectins alone were used. Their preparation has been described (13), as have also the preparation and strength measurement of the gels (4). Stress–strain relations were obtained by means of a Delaware jelly-strength tester (14). The plunger was graduated so that the total stress, expressed as centimeters of water pressure, could be measured as a function of the depression of the plunger into the gel surface. Stress–strain cycles were determined at a constant rate of stress. The ratio, total pressure/plunger depression (tan θ in table 8), is proportional to the elastic modulus of the gel. No attempt was made to define this quantity more rigorously, since the required information regarding the mechanical behavior could be gained from the elastic modulus thus defined, without further mathematical treatment.

Solubility measurements

Dilute (about 4 per cent) solutions of pectinic acids of various λ, both acid and enzyme deësterified, were dried slowly in a convection oven at 80°C. Resulting films were ground and sieved through an 80-mesh screen. To measure the solubility of these materials, the neutralization equivalent was first determined by potentiometric titration with 0.01 *N* sodium hydroxide. Two-gram samples were shaken for 2 hr. with 100 g. of water in a water bath at constant temperature. The mixture was centrifuged, and the concentration of dissolved pectin then determined by titration with 0.01 *N* sodium hydroxide. The weight of dissolved pectin was calculated from the formula relating the weight of pectin to the neutralization equivalent of the sample.

Electrophoresis

Mobility patterns were obtained with a Tiselius–Klett apparatus equipped with the schlieren-scanning device of Longsworth (9). The water bath was maintained at 0.5°C. Five-tenths per cent pectin solutions in 0.1 *N* sodium acetate + 0.01 *N* acetic acid buffer were dialyzed against the buffer for 2 days. Two changes of the buffer were made during the dialysis.

RESULTS AND DISCUSSION

Association and gel formation in the absence of calcium ions

Gel strength and rigidity, and stability toward syneresis are closely related to the solubility of the pectinate in the gel medium. Interpretation of the gelling tendency of pectic materials may be better understood if the solubility is considered both when the sample is a dry powder or film and also when a large proportion of solvent (a gel mixture) is initially present.

TABLE 2

*Solubility of partially soluble pectinic acids in water at 27°C. in the presence of solid phase**

SAMPLE	WEIGHT OF SAMPLE	SOLUTION A: CONCENTRATION OF DISSOLVED PECTINIC ACID	SOLUTION A DILUTED 1 TO 10 AND REËQUILIBRATED WITH SOLUTE: CONCENTRATION OF PECTINIC ACID
	grams	*grams per liter*	*grams per liter*
H84G	12.18	1.569	0.167
H91D	14.798	4.69	0.487
H91E	1.722	0.349	0.033

* Pectinic acids of the weight listed in the second column were shaken for 2 hr. with 1 liter of water at 27°C.

Solubility behavior exhibited by a high polymer such as pectinic acid differs fundamentally from that of a simple substance like sodium chloride. A saturated solution of salt in the presence of solid salt is in thermodynamic equilibrium. If water is added, sufficient salt dissolves to saturate the solution again. In other words, the concentration of dissolved salt attains the same equilibrium value upon dilution when excess solid is present. If an equal volume of water is added to such a system and reëquilibrated, the amount of salt in solution is twice that in the original solution.

A solution of a slightly soluble pectinic acid with excess solid does not act in this manner. Upon addition of solvent no more pectin dissolves, so that the concentration decreases in the same ratio as the dilution increases (table 2). It is evident that the soluble portion of the pectinic acid is *leached out* by the water. Pectinic acid behaves thus because dispersion and solution of its aggregates is a mechanical process of prying apart the molecules that compose them. Water must be able to enter interstices of the aggregates and so solvate the pectinic acid molecules that swelling and eventual solution can occur. Solubility behavior of this sort depends upon the molecular rigidity, the perfection of spatial

ordering, and the magnitude of intermolecular forces. The crystalline phase of a high polymer is usually less soluble than the amorphous phase. In the crystalline regions the chains of a linear polymer like pectinic acid are well aligned, and the maximum number of cross-bonds (hydrogen bonds) per unit length of the molecule is formed. If the molecules are long and rigid (and experimental evidence supports this view (8, 10)), a considerable amount of work is required to separate them, because nearly all the hydrogen bonds between chains must be ruptured simultaneously. For this reason, pectic substances with low λ, and consequently a high concentration of strongly interacting carboxyl groups, tend to crystallize upon precipitation or drying and form relatively insoluble aggregates.

If there are bulky side groups, or if irregularities of a sufficiently high degree are present in the polymer chains, a lesser extent of crystallization results, and

TABLE 3

Solubility of acid-deësterified pectinic acids

Two-gram samples shaken with 100 g. water for 2 hr. at 27°C. and 100°C.

SAMPLE	ASH	λ	BALLAST CONTENT	AMOUNT DISSOLVED AT 27°C.	AMOUNT DISSOLVED AT 100°C.
	per cent		*per cent*	*per cent*	*per cent*
H91	0.25	0.75	22.2	100	100
H91A	0.22	0.57	15.0	100	100
H91C	0.20	0.35	14.5	100	100
H91D	0.40	0.24	8.2	31	100
H91E	0.53	0.11	4.7	20	50
H91K	0.37	0.03	0.9	5	39

aggregates that admit solvent freely are formed. Such aggregates contain a higher proportion of material that can be leached out. Examples are pectinic acids with high λ and high non-galacturonide or "ballast" content (12).

Data of tables 3 and 4 show that acid deësterification, which simultaneously removes ballast and methyl ester groups, effects a greater decrease in solubility than does enzyme deësterification, which leaves the ballast content essentially unchanged. A high content of electrolyte such as sodium chloride promotes solubility. Preparations H88F and H89F, containing about 5 per cent ash, were readily soluble although almost completely deësterified. De-ashing to less than 0.5 per cent reduced their solubility considerably. The electrolyte promotes dissociation of the carboxyl groups and so not only decreases the number of strong hydrogen bonds but creates ionized groups which repel one another. When a solution of a very low ester pectinic acid, relatively free of electrolyte and ballast (H91K), is evaporated slowly, so that extensive hydrogen bridging between carboxyls can take place, the resulting film is crystalline, as revealed by x-ray diffraction, and is so closely packed as not to dissolve completely even in 10 per cent sodium hydroxide.

One of the practical factors discouraging the use of very low ester pectinic

acids has been their limited solubility. It is now obvious that control of the degree of lateral interaction among the pectinic acid molecules, by choice of enzyme rather than acid deësterification, by incorporating electrolyte or other diluent, and by rapid precipitation and drying, can largely remove this limitation.

The tendency of pectinic acids to form aggregates and separate from dilute solution is indicated by the optical quality of the solutions. Table 5 shows the dependence of solution turbidity on λ for acid- and enzyme-deësterified pectinic acids. The aggregates formed irreversibly, since turbidity did not disappear on dilution. It is noteworthy that all the enzyme-deësterified materials gave turbid solutions. This is thought to reflect heterogeneity in λ among the molecules, with the result that those of very low λ and correspondingly great tendency to associate (which are present in every solution) precipitate and contribute the observed cloudiness. Lowering the pH increases the turbidity, and the lower λ is, the greater is the effect.

TABLE 4

Solubility of enzyme-deësterified pectinic acids

Two-gram samples shaken with 100 g. water for 2 hr. at 27°C.

SAMPLE	ASH	λ	BALLAST CONTENT	AMOUNT DISSOLVED AT 27°C.	AMOUNT OF DE-ASHED MATERIAL* DISSOLVED AT 27°C.
	per cent		*per cent*	*per cent*	*per cent*
H91F	0.31	0.55	18.3	100	
H91G	0.51	0.45	17.4	100	
H91H	0.55	0.33	19.7	100	
H91I	0.62	0.23	16.4	100	100
H91J	1.01	0.14	16.2	100 (cloudy)	85 (cloudy)
H88F	4.69	0.03	21.5	100	70 (about)
H89F	4.95	0.04	19.1	100	74

* De-ashed to 0.5 per cent.

Roughly paralleling the turbidity is the viscosity behavior, for with decreasing λ a pronounced viscosity maximum appears near pH 1.5 (figure 1). The origin of both pH effects is attributed to an increase in the number of undissociated carboxyl groups. The viscosity maxima observed for high λ pectinic acids at pH values above 4 are due to electrokinetic effects, since the maxima disappear when 0.1 *N* sodium chloride is present. The low pH maxima persist in 0.1 *N* sodium chloride, so cannot have their origin in electrokinesis.

The formation of pectin gels in concentrated solutions of sugar, glycerol, and glycol at pectin concentrations of less than 1 per cent is often interpreted as a precipitation of the pectin due to preferential solvation of the water by the polyhydroxy compounds. Although, thermodynamically, the addition of a gelling agent may lead to enough change in the activity of the polymer to cause precipitation, it is unlikely that in the case of pectin the mechanism of stable gel formation is one of dehydration alone. It is more probable that sucrose and similar compounds form bridges between the rather stiff pectin molecules (8, 10)

TABLE 5

*Turbidity of 4 per cent aqueous solutions of pectinic acid at 15°C.**

SAMPLE	λ	TURBIDITY†
Acid deësterification		
H91	0.75	−
H91B	0.57	−
H91C	0.35	−
H91D	0.24	++
H91E	0.11	+++
H91K	0.03	++++
Enzyme deësterification		
H91F	0.55	+
H91G	0.45	+
H91H	0.33	++
H91I	0.23	++
H91J	0.14	++

* Partially soluble samples were either heated to dissolve them or partially neutralized and the electrolyte then removed by passage through ion-exchange columns. The solutions were filtered hot and then kept overnight in a refrigerator.

† Turbidity estimated by visual observation. Solutions marked − were clear.

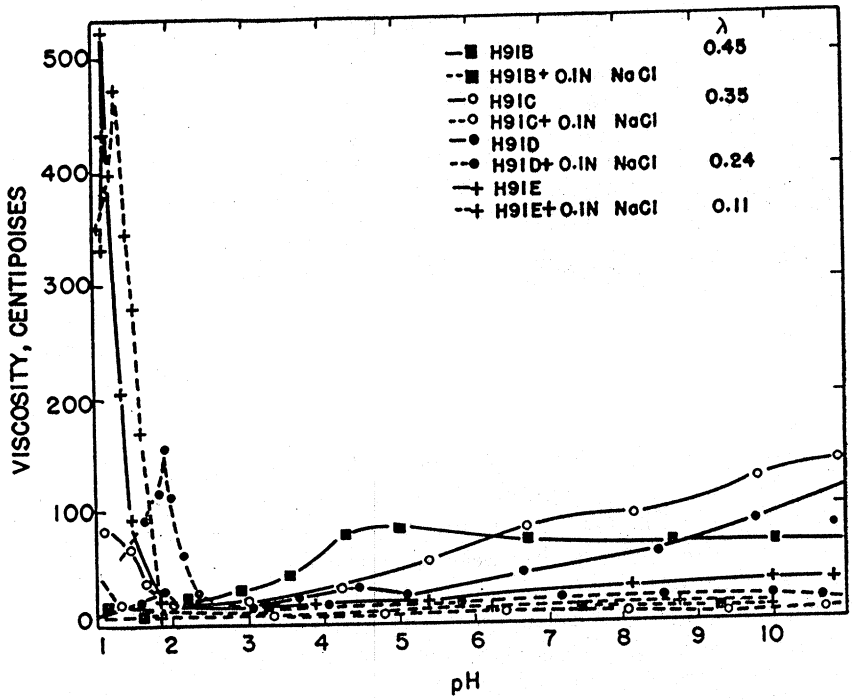

FIG. 1. Effect of pH on the viscosity of acid-deësterified pectinic acids, in the presence and absence of 0.1 *N* sodium chloride. Concentration of pectin, 1 per cent. Measurements made at 12 R.P.M. with No. 2 rotor of Brookfield viscosimeter.

and are able to stabilize a gel structure because of the large total number of hydrogen-bonding groups they present. As λ is decreased, the number of pectin carboxyl groups that can contribute to hydrogen bonding increases, making the effectiveness of the added hydrogen-bonding agents greater (4). Less sugar is then necessary to produce a gel. At very low λ the tendency of pectinic acid to associate with itself is so great that considerable syneresis of the gels takes place.

Sugar gels of high λ when immersed in a large volume of water dissolve from the surface, the gels retaining their cohesion and shape until completely dissolved. Gels of low λ act similarly at pH's greater than about 2. At lower pH's the gels do not dissolve, but the sugar diffuses out, leaving behind the skeleton gels of insoluble pectin.

TABLE 6

*Effect of methyl ester content of pectinate on optimum calcium-to-pectinate ratio and gel strengths of gels made from enzyme- and acid-deësterified pectinic acids**

CH_3O	GEL STRENGTH	CALCIUM/PECTINATE RATIO AT OPTIMUM	METHOD OF PREPARATION
per cent	*cm.* H_2O		
8	16	0.040	Enzyme
7	26	0.020	Enzyme
6	33	0.019	Enzyme
5	37	0.017	Enzyme
7.7	8	0.073	Acid
6.1	270	0.070	Acid
4.9	180	0.038	Acid
4.4	110	0.029	Acid
3.8	84	0.023	Acid

* Data from Hills, White, and Baker (4).

Solubility of pectinic acids in the presence of calcium ions

It has been demonstrated that the ability of dilute pectin solutions to form calcium gels, both in the presence and in the absence of hydrogen-bonding agents, depends upon λ (2, 3, 4). In 1 per cent solution, pectins with λ greater than about 0.5 do not gel with calcium ion alone. When λ is between about 0.5 and 0.25, stable calcium gels are formed, whereas when λ is less than 0.25, the tendency to synerize is great. The lower λ is, the smaller is the amount of calcium ion required to form the gel of optimum strength. Otherwise expressed, the "calcium tolerance" decreases with decreasing λ. This is illustrated by table 6. In this table, per cent methoxyl has been used instead of λ, since carboxyl contents necessary for computation of λ are not available.

Viscosity changes with λ and calcium-ion concentration much as does gelling power. Figures 2 and 3 give data, respectively, for acid- and enzyme-deësterified pectinic acids. Precipitation of calcium pectinate causes the viscosity decrease at the right of the maxima. In practical gel-making, less calcium ion than corresponds to the maxima would be used to avoid cloudiness and syneresis.

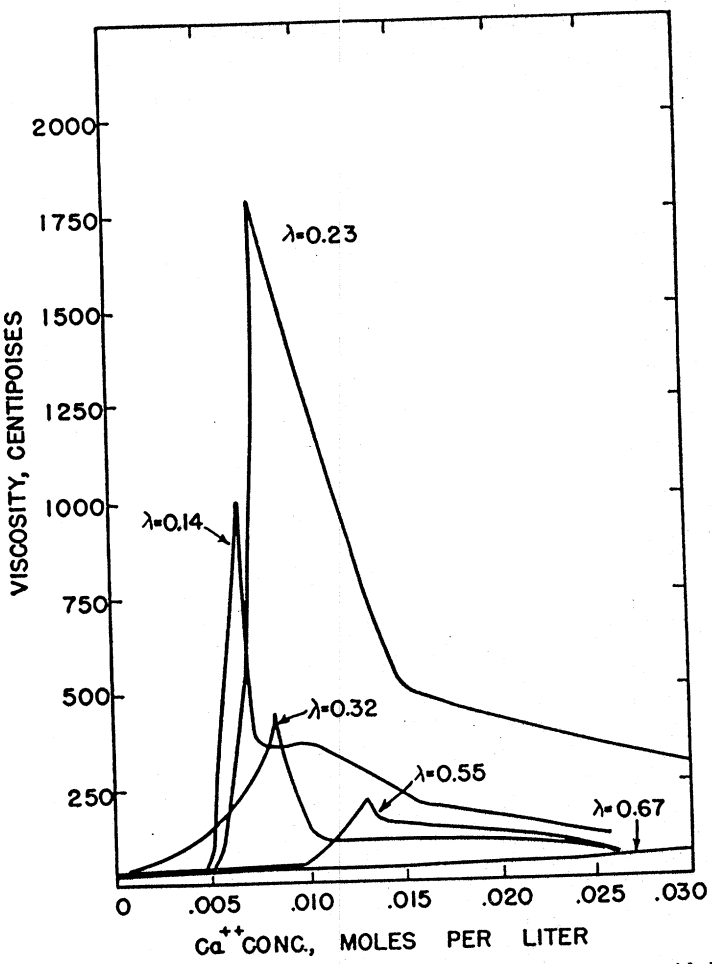

FIG. 2. Effect of calcium chloride concentration on the viscosity of acid-deësterified pectinic acids. Concentration of pectin, 0.25 per cent. Measurements made at 12 R.P.M. with No. 3 rotor of Brookfield viscosimeter.

If the mass-action law is followed, the equilibrium among calcium ion, hydrogen ion, pectinic acid, and calcium pectinate should be expressed by the equation

$$\frac{(Ca^{++})}{(\text{calcium pectinate})} = \frac{K_2}{K_1^2} \frac{(H^+)^2}{(\text{pectinic acid})^2} \qquad (1)$$

in which K_1 is the dissociation constant for pectinic acid (treated as a monobasic acid), and K_2 is the dissociation constant for calcium pectinate. The equation indicates an increase in the calcium tolerance of pectinic acid solutions as the pH is lowered. This expectation is realized experimentally. Table 7 shows for a typical pectinic acid, H91C, that equation 1 describes the equilibrium up to the gel point, since the ratio K_2/K_1^2, is approximately constant to this point.

An explanation of the decreased calcium-ion tolerance with decreasing λ may be sought in the graphs of pH *versus* total calcium-ion concentration in figure 4. It is evident that for equal additions of calcium ion, the lower λ is, the greater is

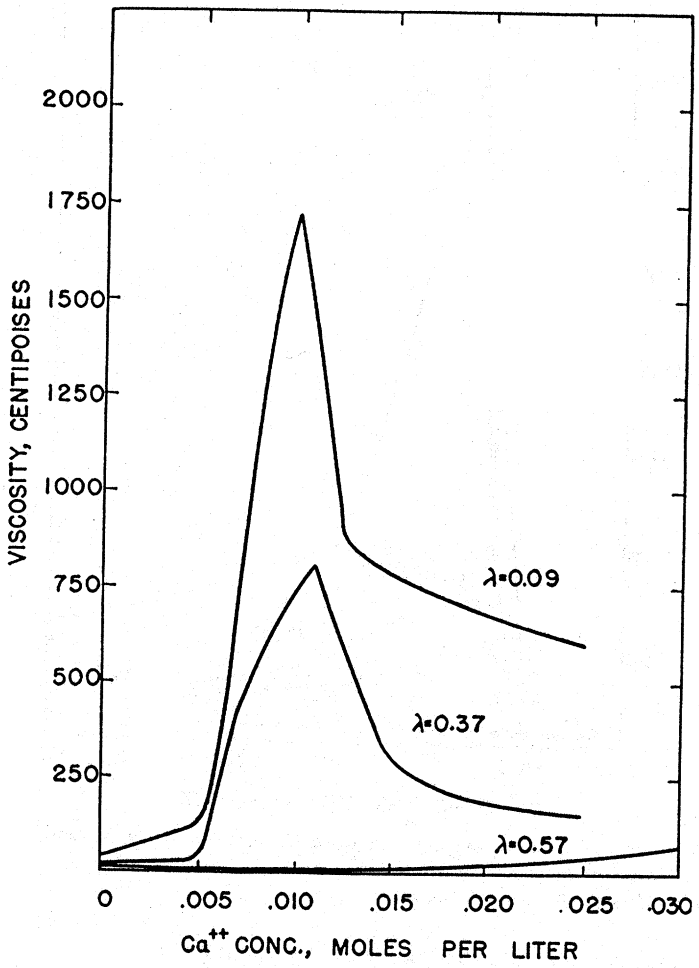

FIG. 3. Effect of calcium chloride concentration on the viscosity of enzyme-deësterified pectinic acids. Concentration of pectin, 0.25 per cent. Measurements made at 12 R.P.M. with No. 3 rotor of Brookfield viscosimeter.

the drop in pH. This means that there is a progressive increase in the amount of bound calcium ion and consequent liberation of hydrogen ion.

The nature of the interaction between pectinic acid and calcium ion is further revealed by the following observations. A calcium pectinate gel incorporating no hydrogen-bonding agent was not visibly altered by 72 hr. immersion in 15 volumes of distilled water. However, when sodium oxalate was added to the water, the calcium ions which linked the pectinic acid molecules were removed

by precipitation as the oxalate ions diffused into the gel, and the gel dissolved. Evidently the calcium ion is so firmly bound to the carboxyl groups that it cannot diffuse out of the gel, and since osmotic pressure is insufficient to force more water into the gel, the calcium-ion concentration within the gel is unchanged, and the gel is stable even in the presence of a considerable excess of solvent. In this respect, calcium gels are different from sugar gels. Oxalate ions are able to move fairly freely into the gel and, by sequestering much of the calcium, promote its dissolution. Like the oxalate, but less effectively, hydrogen ions can also remove calcium from the gel network, with the result that it disintegrates and dissolves.

TABLE 7

Calcium pectinate–pectinic acid equilibrium (sample H91C)

TOTAL Ca^{++} ADDED	INCREASE IN (H^+)*	$\frac{K_2}{K_1^2}$
moles per liter	*moles per liter*	
3×10^{-4}	0.91×10^{-4}	51.5
6.01	1.37	48.5
9.01	1.79	52.3
12.02	2.35	47.2
18.03	2.71	60.9†
36.06	3.99	68.0
54.09	4.92	71.5
84.14	5.91	79.4
120.20	6.22	110.0

* Calcium pectinate concentration assumed equal to $\frac{1}{2}\Delta(H^+)$.

† Gelation occurred at this point.

Charge distribution and gel formation

As stated earlier, the inferior strength of enzyme-deësterified calcium pectinate gels was thought to reflect a peculiar distribution in the degree of esterification among the pectinate molecules (2), that is, because the action of pectinesterase may be highly selective, some molecules of a pectinic acid sample are demethylated to a very different degree from others. Acid and alkali presumably remove ester groups at random, so that λ for each individual molecule is about the same as the average λ for the entire preparation. Enzyme deësterification would then produce a broad distribution in λ, as compared with acid deësterification.

The electrophoretic mobility of a pectin molecule is proportional to $\alpha(1 - \lambda)$, where α is the degree of dissociation. Heterogeneity in λ should then be revealed in electrophoresis patterns. Figures 5 and 6, for acid- and enzyme-deësterified pectinic acids, show the expected broader distribution in mobility in the enzyme-treated preparations. Owens (7) recently has made similar observations on citrus pectin. He has fractionated enzyme-deësterified pectins by chemical means, and has found marked differences in λ among the fractions.

Ascending boundaries in figure 6, D and E, obtained from enzyme-deësterified

pectinic acid, demonstrate the presence of a small amount of a second component. The mobility of the second component is greater in figure 6D and less in figure 6E than the mobility of the principal component. We have also observed ascending boundaries in which three components were present. This is apparently a consequence of the non-random nature of pectin demethylation by enzymes.

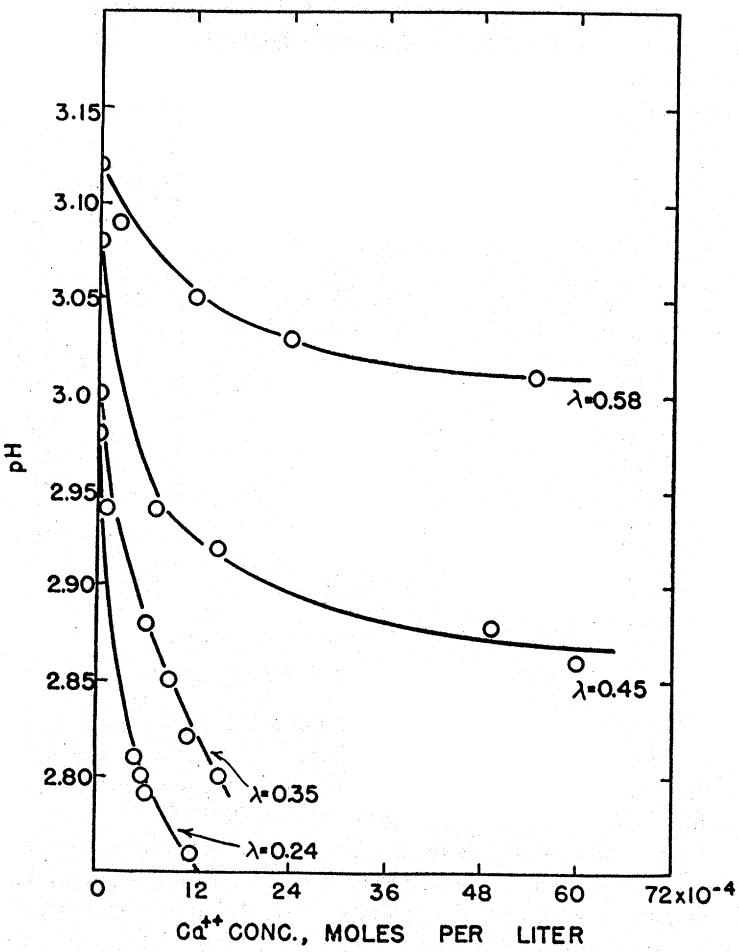

FIG. 4. Effect of calcium chloride on the pH of pectinic acids of various λ. Concentration of pectin, 0.2 per cent.

Explanation of the difference in gel strength between 65 per cent sugar and 35 per cent sugar–calcium pectinate gels shown in tables 1 and 6 can now be based on two experimental facts: (*1*) The amount of calcium ion required for maximum strength decreases with λ. (*2*) For a given λ all the molecules are not deësterified to the same extent, the deviation from the average being much greater in enzyme- than in acid-deësterified pectinic acids. Consequently, since nearly all the molecules have the same λ, a single calcium concentration is

optimum for an acid-deësterified pectinic acid. However, a single calcium concentration is not optimum for all the molecules of an enzyme-deësterified pectinic acid. The fraction of low λ requires less calcium than the fraction of high λ, so an intermediate calcium concentration would precipitate the former

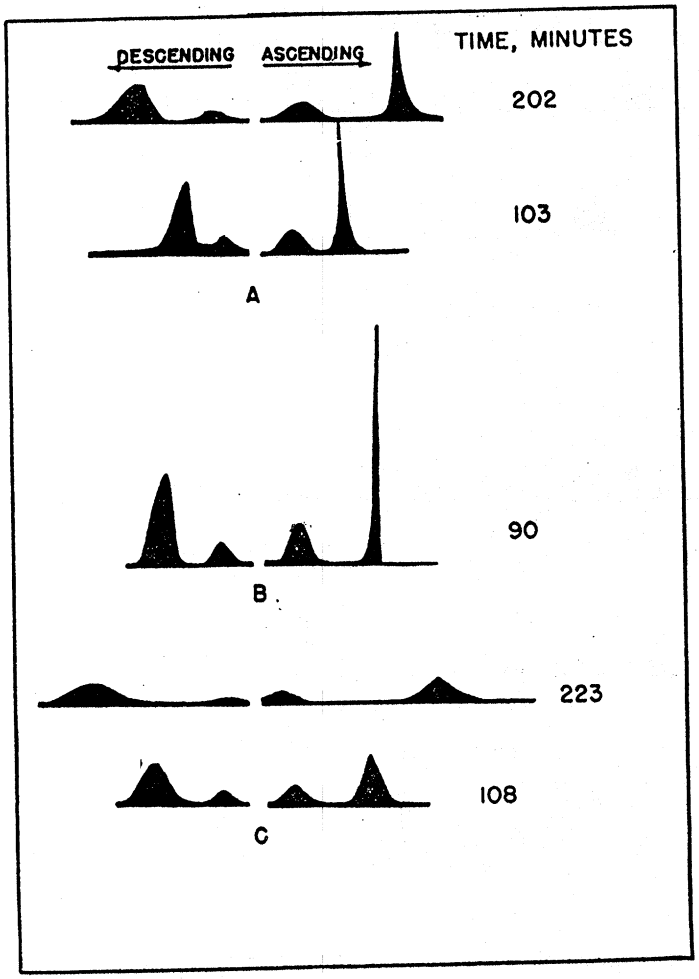

FIG. 5. Electrophoresis patterns of pectic materials
A: H84, the original pectin, λ = 0.80, pH = 5.7
B: H84D, acid deësterified, λ = 0.32, pH = 5.7
C: H89C, enzyme deësterified, λ = 0.35, pH = 5.7

while incompletely cross-linking the latter. This would make gels of low strength. An experiment performed by Hills (2) illustrates the point. Two pectinic acids produced by acid deësterification, with methoxyl contents of 3.3 and 5.7 per cent, gave calcium gels with strengths of 88 and 89 cm. The pectinic acids were mixed in equal amounts. The methoxyl content of the mixture was 4.5 per cent, and the distribution in λ was no longer sharp but resembled that of an enzyme-

deësterified pectin. Significantly, the calcium gel strength was only 44, or half that of the gel made from each constituent of the mixture. A similar mixture made with enzyme-deësterified pectinic acids did not give a correspondingly

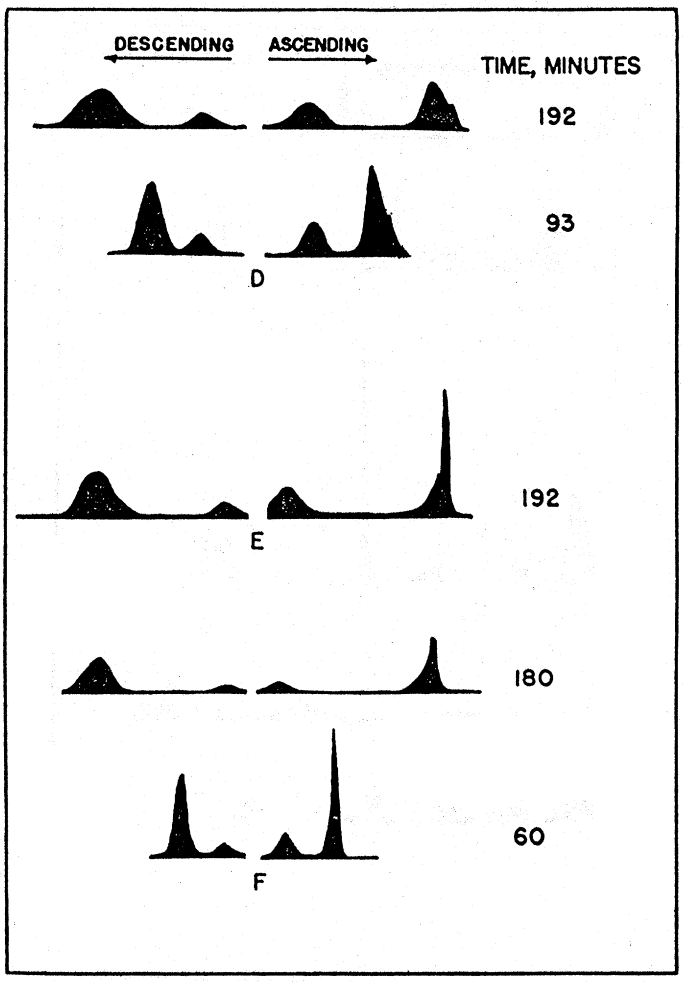

FIG. 6. Electrophoresis patterns of pectinic acids
D: H88C, enzyme deësterified, $\lambda = 0.35$, pH = 5.6
E: H91I, enzyme deësterified, $\lambda = 0.23$, pH = 5.6
F: H59, acid deësterified, $\lambda = ca.\ 0.35$, pH = 5.8

weakened gel. This result was expected, since initially the enzyme-treated pectins were heterogeneous in λ.

As the average λ becomes small, the distribution in λ among the molecules of enzyme-deësterified pectinic acids inevitably becomes more homogeneous, and the gel strength rises until syneresis reverses the trend (table 6). Electrophoresis patterns demonstrate directly the increased homogeneity, Sample H91I with

$\lambda = 0.23$ giving much sharper peaks than enzyme-treated preparations of greater λ.

The possibility should be mentioned that non-random demethylation by an enzyme may result in giving part of a pectinic acid molecule the properties of pectic acid and part the properties of a high-ester pectin. Such a condition would also contribute to the observed differences in solubility, viscosity, electrophoretic mobility, and gel behavior of acid- and enzyme-deësterified pectinic acids, although to a smaller extent than the heterogeneity in λ discussed above. Jansen and MacDonnell (5) have accumulated impressive experimental evidence that hybrid pectic acid–pectin molecules actually result from pectase action.

Influence of molecular weight

The strength of calcium pectinate gels depends upon molecular weight, λ, and the calcium-binding power. During acid deësterification there is simultaneous progressive molecular degradation (12). In a λ series (table 6) the initial strength, which expresses the effect of high molecular weight, is at first exceeded despite degradation, because of the overbalancing effect of the increased number of strong ionic cross-links made possible as λ is lowered. Ultimately, the molecular-weight effect, partly obscured, it is true, by precipitation and syneresis, again predominates, and the gel strength falls. Similar behavior is shown in the viscosity curves of figure 2.

Controlled enzyme deësterification leads to little chain degradation, so the molecular weight is nearly independent of λ (12). Therefore gel strength (table 6) and viscosity (figure 3) increase with decreasing λ. Gel syneresis is here less troublesome because the large content of ballast material represses association.

Bond types and the elastic properties of pectin gels

Observed gel elasticity must arise from bending of the pectin molecules and deformation of three-dimensional interweaving networks of multilaterals of different size and shape made by hydrogen and calcium-ion bridges between portions of the pectin chain.

In 65 per cent sugar gels hydrogen bridges alone are active. Because of the predominant proportion of sugar and the large size and low mobility of its molecules in comparison with water, the bridges between pectin molecules must be principally through sugar. To form a gel, the pectin molecules need nowhere be in contact or even close to one another. Consequently, it might be expected that the sugar gels would be easily deformed and relatively elastic. In calcium pectinate gels the most effective cross-bonds are calcium-ion bonds between carboxyls. Since the bond distances are short, the pectin molecules must approach very closely at these tie points. The bonds are strong and not distensible. An inelastic, rather brittle gel results, as is shown in table 8. Recoverable deformation is only about one-half that of sugar gels, although the strength of calcium pectinate gel may be as great or even greater. Also characteristic of a brittle material are the high values of tan θ and the small work of deformation.

Tan θ, which is analogous to an elasticity modulus, gives an approximately

straight line when plotted against the ultimate strength for a series of sugar gels. Owens and Maclay (9) found that plots of shear modulus *versus* ultimate strength are also linear. This means that the modulus of rigidity is a measure of the strength of a 65 per cent sugar pectin gel, just as it is for gelatin gels (11). There is not even approximate linearity between strength and tan θ for calcium pectinate gels, and this makes an analysis of their mechanical behavior considerably more difficult.

TABLE 8

Mechanical properties of pectin gels

SAMPLE	CH_3O	WEIGHT OF PECTIN	TAN θ§	ULTIMATE STRENGTH	REVERSIBLE DEFORMATION¶
	per cent	*grams*		*cm.* H_2O	*per cent*
Sugar gels (65%):					
E35-1*	8.06	1	32.5	156.0	About 60
-12	8.06	0.6	23.3	112.0	54
-13	8.06	0.6	23.0	114.0	52
H59-11†	4.53	1	17.0	72.0	43
-12	4.53	1	17.2	67.0	40
-14	4.53	2	21.3	150	59
-15	4.53	2	20.0	170	58
H74-18‡	4.50	1	5.6	8	Very low
-19	4.50	1	4.1	11.5	Very low
-21	4.50	2	13.4	47.6	43
-22	4.50	2	13.3	50.5	32
Sugar–calcium pectinate gels (35%):					
H59-1†	4.53	2	50.0	56	25
-2	4.53	2	62.5	48	37

* Unmodified pectin.

† Acid-deësterified pectinic acid.

‡ Enzyme-deësterified pectinic acid.

§ Tan $\theta = \frac{\text{pressure in cm. } H_2O}{\text{displacement in mm.}}$; obtained graphically.

¶ Gel deformed under a stress of 30 cm. H_2O and then allowed to recover at zero load.

$$\text{Per cent reversible deformation} = \frac{\text{reversible deformation} \times 100}{\text{total deformation}}$$

It is worthy of note, finally, that for sugar gels of increasing strength, permanent flow decreases and reversible deformation increases.

SUMMARY

1. The solubility of pectic materials is an index to their gel-forming ability. Solubility in general decreases with the degree of esterification, λ. Because of their higher content of non-uronide material, enzyme-deësterified pectinic acids are more soluble than acid-deësterified pectinic acids.

2. Molecular association in pectinic acid solutions is also expressed by the development of turbidity and heightened viscosity.

3. The quantity of divalent ion (Ca^{++}) necessary to form a gel of a given strength decreases with λ, reflecting the increased possibility of forming cross-links between carboxyl groups of adjacent pectinic acid molecules.

4. Electrophoresis diagrams for acid- and enzyme-deësterified pectinic acids show the latter to be more heterogeneous in the distribution of λ among its molecules. This heterogeneity is principally responsible for the low strength of gels made from enzyme-deësterified pectinic acids.

5. Calcium pectinate gels are characteristically brittle as compared with hydrogen-bonded pectinate gels.

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